MALARIA

A randomized controlled trial showing safety and efficacy of a whole sporozoite vaccine against endemic malaria

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A highly effective malaria vaccine remains elusive despite decades of research. *Plasmodium falciparum* sporozoite vaccine (PfSPZ Vaccine), a metabolically active, nonreplicating, whole parasite vaccine demonstrated safety and vaccine efficacy (VE) against endemic *P. falciparum* for 6 months in Malian adults receiving a five-dose regimen. Safety, immunogenicity, and VE of a three-dose regimen were assessed in adults in Balonghin, Burkina Faso in a two-component study: an open-label dose escalation trial with 32 participants followed by a double-blind, randomized, placebo-controlled trial (RCT) with 80 participants randomized to receive three doses of 2.7×10^6 PfSPZ ($N = 39$) or normal saline ($N = 41$) just before malaria season. To clear parasitemia, artesunate monotherapy was administered before first and last vaccinations. Thick blood smear microscopy was performed on samples collected during illness and every 4 weeks for 72 weeks after last vaccinations, including two 6-month malaria transmission seasons. Safety outcomes were assessed in all 80 participants who received at least one dose and VE for 79 participants who received three vaccinations. Myalgia was the only symptom that differed between groups. VE (1 − risk ratio; primary VE endpoint) was 38% at 6 months ($P = 0.017$) and 15% at 18 months (0.078). VE (1 − hazard ratio) was 48% and 46% at 6 and 18 months ($P = 0.061$ and 0.018). Two weeks after the last dose, antibodies to *P. falciparum* circumsporozoite protein and PfSPZ were higher in protected versus unprotected vaccinees. A three-dose regimen of PfSPZ Vaccine demonstrated safety and efficacy against malaria infection in malaria-experienced adults.

INTRODUCTION

Little change in malaria cases and deaths occurred from 2015 to 2020, when 241 million cases and 627,000 deaths were reported (*1*). New tools are needed to combat malaria and achieve the United Nations sustainable development goal three, to "ensure healthy lives and promote well-being for all at all ages." This ambitious aim targets a 90% reduction in malaria incidence and mortality by 2030 (*2*).

A protective vaccine would represent an important tool to combat the enormous health and socioeconomic burden of malaria. Immunization with radiation-attenuated, metabolically active, nonreplicating, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ), Sanaria PfSPZ Vaccine, has induced >90% protection against controlled human malaria infection (CHMI) in studies in the United States (*3*, *4*), Tanzania (*5*), and Mali (*6*) with protection lasting for at least 8 to 14 months (*4*, *7*, *8*). Malaria-experienced, African adults who received five doses of 2.7×10^5 PfSPZ demonstrated 52% vaccine efficacy (VE) against naturally transmitted infection by hazard ratio (HR) and 29% by risk ratio (RR) analysis (*9*).

To reduce the number of required injections and improve VE, we assessed the safety, tolerability, immunogenicity, and VE of three injections of a 10-fold higher dose of PfSPZ Vaccine in adults living in an area of intense, seasonal *P. falciparum* transmission in Burkina Faso (*10*).

RESULTS

Study population

From 12 April to 3 May 2016, 32 adults enrolled in the dose escalation trial (cohorts 1 to 4), and from 6 to 24 March 2017, 80 enrolled in the randomized trial (cohort 5). For cohorts 1 to 4, 32 participants enrolled, received at least one vaccine dose, and were included in the safety analysis; two missed sampling time points and were excluded from immunogenicity analysis (Fig. 1). For cohort 5, 80 participants enrolled, received at least one vaccine dose, and were included in the safety analysis; one participant assigned to PfSPZ Vaccine received three doses of placebo and was analyzed according to treatment received. Thus, safety and intention-to-treat (ITT) populations included 39 vaccinees and 41 controls (Fig. 1). One placebo recipient missed the third vaccination

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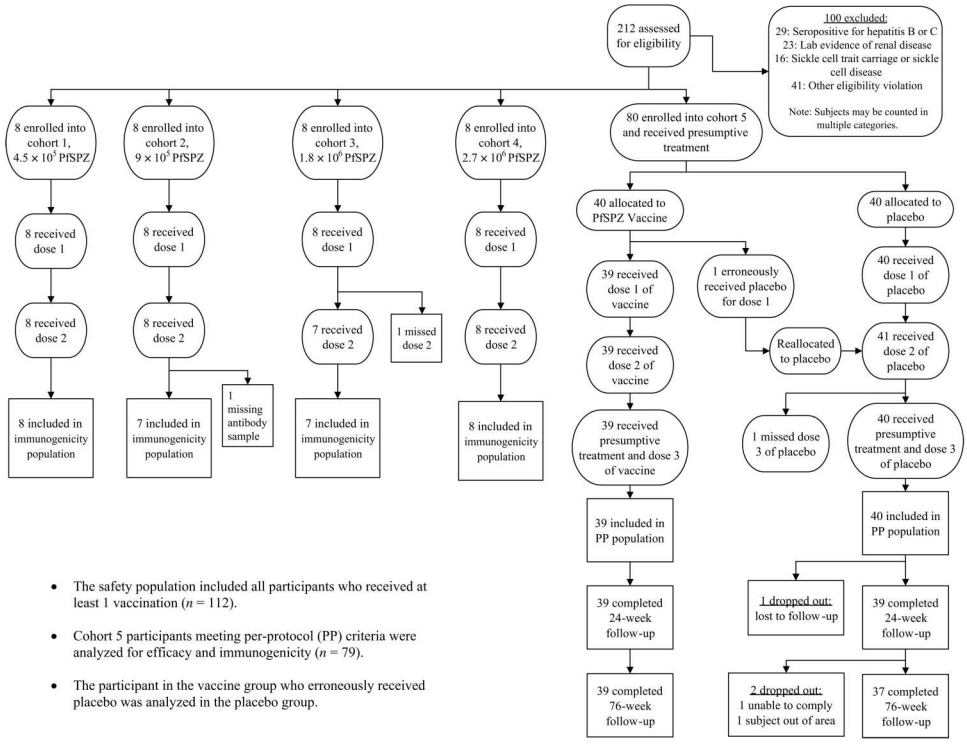


Fig. 1. Trial profile. *P. falciparum*

and was excluded from per-protocol (PP) analyses. Of 79 in the PP population, one control dropped out during the primary VE follow-up period, and two controls dropped out afterward (Fig. 2).

Baseline characteristics were balanced between groups (Table 1). All cohort 5 participants completed treatment with artesunate monotherapy at a mean of 4.9 and 5.1 days before the first and third vaccinations, respectively. All were thick blood smear (TBS) negative at the time of the first and third immunizations. All 39 vaccinees and 39 of 41 (95%) controls were TBS negative throughout the 16 weeks of vaccinations.

Feasibility and safety results

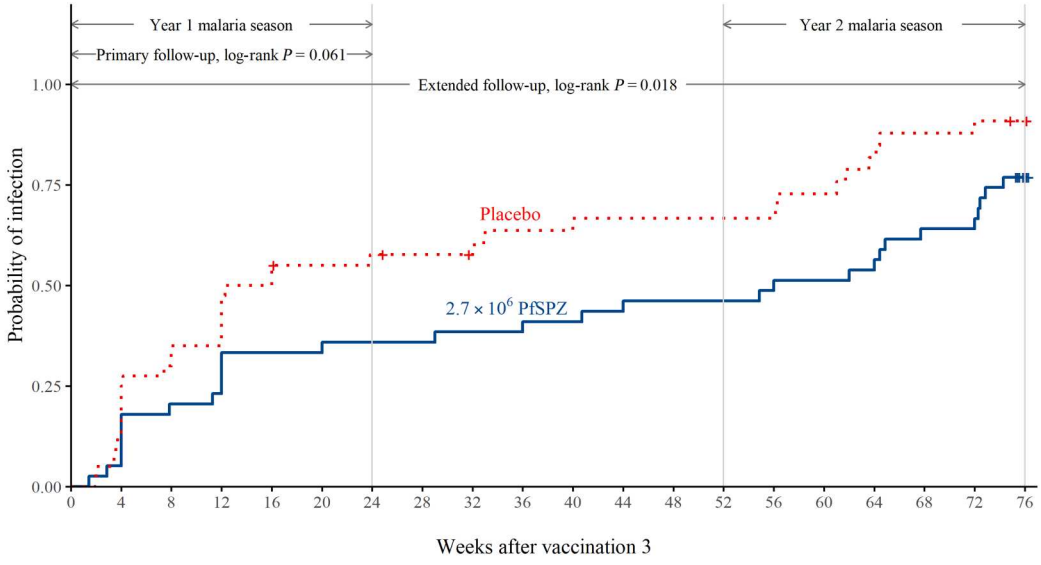
All 302 injections in cohorts 1 to 5 were administered successfully by direct venous inoculation (DVI), generally in less than 5 s, with no partial injections or extravasation noted. Mild, postinjection local pain persisting for 1 day was reported by one vaccinee and one control (Table 2). Most participants reported no solicited adverse events (AEs) during the 7 days after vaccination (Table 2). In cohort 5, 15 (38%) vaccinees and 10 (24%) controls reported systemic AEs. Most common were headache [12 (31%) vaccinees versus 8 (20%) controls], myalgia [7 (18%) versus 1 (2%)], arthralgia [4 (10%) versus 2 (5%)], and nausea [3 (8%) versus 1 (2%)]. Only myalgia was more common in vaccinees ($P = 0.027$, Fisher's exact test). In addition to myalgia, proportions of local and systemic AEs that were at least 10% more common in PfSPZ Vaccine recipients versus placebo controls in cohort 5 include only grade 1 headache in nine vaccinees (23.1%) versus five controls (12.2%), but this difference was not statistically significant. Within 28 days after each vaccination, unsolicited related AEs were experienced by eight participants in cohorts 1 to 4 and three participants (one vaccinee and two controls) in cohort 5. All were mild in severity. The most common were bradycardia, tachycardia, and tachypnea, each experienced by three participants (table S1). Serious AEs were recorded in eight participants: two in cohort 2 and six in cohort 5, including four in the placebo group; all were considered unrelated to the study product (table S2). Laboratory abnormalities occurred after vaccination in 16% of participants and, in cohort 5, did not differ between vaccinees and controls (table S3, A and B). Grade 1 elevations of alanine aminotransferase (ALT) (61.1 to 121.9 IU/liter) were the most commonly encountered laboratory abnormality in cohort 5 vaccinees (table S3B).

VE against endemic malaria

We analyzed occurrence and time to first Pf infection for cohort 5 participants during the 24-week malaria transmission season that started after the third vaccinations and for an extended period covering 76 weeks through two malaria transmission seasons with a dry season in between (Fig. 2). Infection was measured by TBS microscopy on samples collected every 4 weeks and during illness.

Twenty-four–week follow-up

Twenty-three of 40 (58%) controls became positive compared with 14 of 39 (36%) vaccinees in the PP population, giving an overall 37.7% VE by the RR method [95% confidence interval (CI), 6.8 to 68.7%; $P = 0.017$]. Although all efficacy endpoints are exploratory, this one was prespecified as the primary efficacy endpoint. VE was also calculated as 1 − HR to facilitate comparison to other studies; this was 47.9% (95% CI, −2.9 to 73.6%; $P = 0.061$). This result was similar when the HR was adjusted for covariates (47.0% VE; 95% CI, −6.5 to 73.6%; $P = 0.075$).



Cumulative events

2.7 × 10^6 PfSPZ	0	7	8	13	13	14	14	14	15	16	16	18	18	18	20	20	22	25	26	30
Placebo	0	10	14	19	22	22	23	23	23	25	26	26	26	26	26	28	31	33	34	34

Cumulative symptomatic events

2.7 × 10^6 PfSPZ	0	2	3	4	4	4	4	4	4	4	4	4	4	4	6	6	6	6	6	6
Placebo	0	6	8	9	9	9	10	10	10	10	10	10	10	10	10	11	12	12	13	13

Cumulative asymptomatic events

2.7 × 10^6 PfSPZ	0	5	5	9	9	10	10	10	11	12	12	14	14	14	14	14	16	19	20	24
Placebo	0	4	6	10	13	13	13	13	13	15	16	16	16	16	16	17	19	21	21	21

Vaccine efficacy (VE) estimates:

	Primary follow-up (0–24 weeks)		Extended follow-up (0–76 weeks)	
VE method	VE [95% CI]	*P* value	VE [95% CI]	*P* value
1 – risk ratio	0.377 [0.068, 0.687]	0.017	0.154 [–0.017, 0.325]	0.078
1 – hazard ratio (HR)	0.479 [–0.029, 0.736]	0.061	0.459 [0.101, 0.675]	0.018
1 – adjusted HR	0.470 [–0.065, 0.736]	0.075	0.449 [0.073, 0.672]	0.025

HRs were calculated using Cox regression. The adjusted model included log-transformed baseline antibody level and age.

Fig. 2. Efficacy of PfSPZ Vaccine against naturally occurring infection. Inverse survival curves for the time after the last vaccination to the first positive TBS (asymptomatic + symptomatic infection). Efficacy was analyzed as 1 − RR ("proportional analysis") and 1 − HR for the primary follow-up period (0 to 24 weeks after the third vaccination) and the extended follow-up period (0 to 76 weeks after the third vaccination). The survival curves include 79 participants who received all three vaccinations and were evaluable for the VE endpoint. The Kaplan-Meier estimated RRs are the ratio of the height of the blue solid curve to the height of the red dashed curve at 24 and 76 weeks.

Seventy-six–week follow-up

Thirty-four of 40 (85%) controls became positive, and 30 of 39 (77%) vaccinees became positive, giving an overall 15.4% VE by the RR method (95% CI, −1.7 to 32.5%; P = 0.078). VE by the HR method was 45.9% (95% CI, 10.1 to 67.5%; P = 0.018). This result was similar when the HR was adjusted for covariates (44.9% efficacy; 95% CI, 7.3 to 67.2%; P = 0.025).

Antibody responses

Antibody responses were assessed at baseline and at 2 weeks after last vaccinations. We analyzed additive changes from baseline (referred to as "net") and multiplicative changes from baseline ("ratios"; tables S4 and S5).

Pf circumsporozoite protein (enzyme-linked immunosorbent assay)

Dose escalation component (cohorts 1 to 4). Participants in cohorts 1 to 3 showed similar responses after two doses. Cohort 4 participants showed greater antibody responses compared to cohorts 1 to 3 (Fig. 3A and fig. S1A).

Randomized controlled trial (cohort 5). Antibody responses [net optical density (OD) 1.0] were higher among vaccinees than controls (P < 0.001; Fig. 3B and fig. S2), as were ratios (P < 0.001; fig. S1B); 28 of 39 (72%) vaccinees and 0 of 39 (0%) controls seroconverted (P < 0.001; table S6).

Association with 24-week VE. Antibodies to Pf circumsporozoite protein (PfCSP; net OD, 1.0) were higher (median net OD 1.0, 5550 versus 1410) among uninfected (protected) than infected vaccinees (P = 0.01; Fig. 3B), as were OD 1.0 ratios (P = 0.016; fig. S1B and table S7).

Table 1. Baseline characteristics of participants who received at least one vaccination.

	Cohort 1: 4.5×10^5 PfSPZ Vaccine (n = 8)	Cohort 2: 9×10^5 PfSPZ Vaccine (n = 8)	Cohort 3: 1.8×10^6 PfSPZ Vaccine (n = 8)	Cohort 4: 2.7×10^6 PfSPZ Vaccine (n = 8)	Cohort 5: 2.7×10^6 PfSPZ Vaccine (n = 39)	Cohort 5: placebo (n = 41)	Total (N = 112)
Sex							
Male	2 (25%)	7 (88%)	4 (50%)	5 (63%)	21 (54%)	23 (56%)	62 (55%)
Female	6 (75%)	1 (13%)	4 (50%)	3 (38%)	18 (46%)	18 (44%)	50 (45%)
Age, years							
Mean (SD)	35.1 (2.7)	29.6 (4.9)	32.1 (4.8)	28.6 (5.2)	30.7 (5.4)	30.3 (5.5)	30.8 (5.3)
Range	31–39	23–35	26–38	22–39	21–40	21–40	21–40
Malaria infection by TBS at enrollment							
***n* (%)**	0 (0%)	2 (25%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	3 (3%)
Anti-PfCSP antibodies by ELISA (OD 1.0)							
Median	1411.5	918.5	583	700	1009	768	836
Range	647–8491	266–2018	278–2267	478–4742	162–7096	133–2974	133–8941
Anti-PfSPZ antibodies by automated immunofluorescence assay (AFU 2×10^5)							
Median	Not collected for these cohorts				439	474	460
Range					0–2967	0–1283	0–2967
Anti-PfSPZ antibodies by inhibition of sporozoite invasion assay (reciprocal serum dilution for 80% inhibition)							
Median	Not collected for these cohorts				14.48	13.96	14.17
Range					0–90.39	0–69.46	0–90.39

PfSPZ (automated immunofluorescence assay). Antibody responses to PfSPZ [net arbitrary fluorescence units (AFU)] were higher among vaccinees than controls ($P < 0.001$; Fig. 3C), as were AFU ratios ($P < 0.001$; fig. S1C); 24 of 39 (62%) vaccinees and 1 of 39 (3%) controls seroconverted ($P < 0.001$; table S6).

Association with 24-week VE. Antibodies to PfSPZ were higher among uninfected than infected vaccinees ($P = 0.016$; Fig. 3C and table S5), but the differences in AFU ratios did not reach statistical significance ($P = 0.08$; fig. S1C); 19 of 25 (76%) uninfected and 5 of 14 (36%) infected vaccinees seroconverted ($P = 0.015$; table S7).

Functional activity (automated inhibition of SPZ invasion assay)

The functional activity was higher among vaccinees than controls ($P < 0.001$; Fig. 3D), as were the 80% inhibition ratios ($P = 0.002$; fig. S1D); 21 of 39 (54%) vaccinees and 9 of 40 (22%) controls seroconverted ($P = 0.004$; table S3).

Association with 24-week VE. Net 80% inhibitory activity ($P = 0.891$) and ratios ($P = 0.361$) were not different between uninfected and infected vaccinees (Fig. 3D and fig. S1D); 13 of 25 (52%) uninfected and 8 of 14 (57%) infected vaccinees seroconverted ($P = 0.798$; table S7).

DISCUSSION

PfSPZ Vaccine is a whole eukaryotic cell vaccine. No whole eukaryotic cell vaccine with marketing authorization (licensure) exists for any infectious agent, and no vaccine with marketing authorization exists for human disease caused by any parasite, including Pf. Thus, no precedent describes how to develop or finalize a vaccination regimen for PfSPZ Vaccine, and development has required an empirical approach.

Five doses of 2.7×10^5 PfSPZ were protective against Pf infection in Mali (*9*). In parallel with this trial, a regimen of three doses of 1.8×10^6 PfSPZ in Malian adults showed similar protection to the five-dose regimen (*6*), whereas VE against CHMI in Tanzanian adults receiving three doses of 1.8×10^6 PfSPZ versus 9.0×10^5 PfSPZ demonstrated that the lower dosage regimen was superior (*5*). As a part of the overall program to determine an optimal dose regimen, we used a three-dose regimen in this trial for which each dose was 10 times greater than the five doses assessed in Mali and 50% greater than those in the three-dose regimen in Mali. This three-dose regimen was easy to administer, well tolerated, and showed no serious safety concerns that would suspend further development. It induced VE for 18 months and antibodies against PfCSP and PfSPZ that correlated with protection.

PfSPZ Vaccine is administered by DVI of 0.5 ml through a 25-gauge needle for several seconds, a procedure that is generally simple and painless in adults (*4*, *9*, *11*, *12*) and was not associated with crying in 16% of 1067 injections of infants in Kenya (*13*). In this trial, 302 injections by DVI were administered without complication. Despite initial concerns about vaccination by DVI, data from multiple clinical trials indicate that there is no difference in success rates between children (≥2 years of age) and adults (*5*, *14*). Interest in immunization by DVI has recently increased (*15*).

The overall frequency of AEs was low, and in all cases but one, rates of AEs did not differ between vaccinees and NS controls, consistent with the results of numerous published studies (*5*, *9*, *12*–*14*, *16*–*22*) of PfSPZ Vaccine. Unique to this trial, the frequency of myalgia in the 7 days after vaccination was statistically significantly

Table 2. Solicited local and systemic AEs after vaccination. For cohort 5 participants, the only significant difference in solicited symptoms was for myalgia, which was more commonly reported in PfSPZ Vaccine versus placebo recipients (18% versus 2%, $P = 0.027$), and all mild in severity.

	Cohort 1: 4.5×10^5 PfSPZ Vaccine ($n = 8$)	Cohort 2: 9×10^5 PfSPZ Vaccine ($n = 8$)	Cohort 3: 1.8×10^6 PfSPZ Vaccine ($n = 8$)	Cohort 4: 2.7×10^6 PfSPZ Vaccine ($n = 8$)	Cohort 5: 2.7×10^6 PfSPZ Vaccine ($n = 39$)	Cohort 5: placebo ($n = 41$)	Total ($N = 112$)
Local AEs							
Ecchymosis/bruising							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Erythema/ redness							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Induration/swelling							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pain at injection site							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	1 (2.4%)	2 (1.8%)
Tenderness at injection site							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Any local symptom							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	1 (2.4%)	2 (1.8%)
Systemic AEs							
Arthralgia/joint pain							
Grade 1	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	1 (2.4%)	3 (2.7%)
Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (7.7%)	1 (2.4%)	4 (3.6%)
Chills							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.4%)	1 (0.9%)
Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	0 (0%)	1 (0.9%)
Headache							
Grade 1	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	9 (23.1%)	5 (12.2%)	15 (13.4%)
Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (7.7%)	3 (7.3%)	6 (5.4%)
Malaise							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Myalgia/body aches							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 (17.9%)	1 (2.4%)	8 (7.1%)
Nausea							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (7.7%)	1 (2.4%)	4 (3.6%)
Fever							
Grade 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.6%)	0 (0%)	1 (0.9%)
Any systemic symptom							
Grade 1	1 (12.5%)	0 (0%)	1 (12.5%)	0 (0%)	10 (25.6%)	6 (14.6%)	18 (16.1%)
Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (12.8%)	4 (9.8%)	9 (8.0%)

higher than in the placebo group (18% versus 2%, Fisher's exact test $P = 0.027$), although the clinical importance of this difference is not clear. In Equatorial Guinea, the same dosage regimen of PfSPZ Vaccine was assessed, and myalgia was not significantly increased (*19*). The frequencies of headache and grade 1 ALT elevation in the current trial were numerically higher but not statistically significant. In published, randomized, placebo-controlled, double-blind trials of PfSPZ Vaccine in which 325 adults received PfSPZ Vaccine and 175 adults received normal saline (NS) placebo, there were no significant differences in the frequencies of either myalgias or headaches (*5*, *6*, *9*, *12*, *14*, *17*–*22*). Although the increase in myalgias may be a characteristic of the population studied, we believe it is more likely a consequence of small numbers of participants. In the same nine trials, only three grade 2 ALT or aspartate aminotransferase (AST) elevations in 325 vaccinees (0.92%) and one grade 2 ALT elevation in 175 NS control subjects (0.57%) were identified, indicating that hepatocellular injury is very unlikely caused by PfSPZ Vaccine.

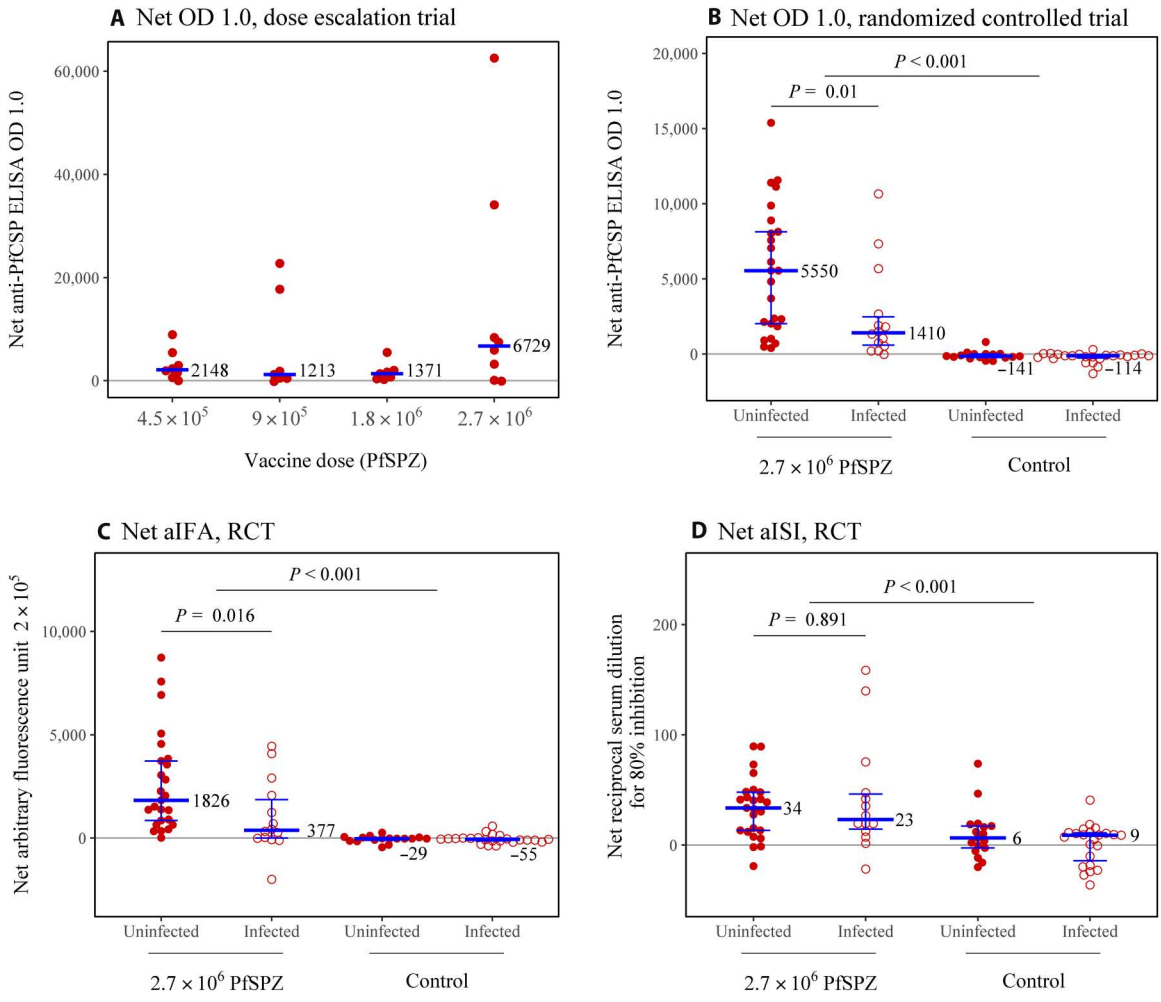


Fig. 3. Antibody responses. Antibody responses to PfCSP were measured before immunization and at 2 weeks after the last dose of PfSPZ Vaccine or placebo (second dose for cohorts 1 to 4 and third dose for cohort 5, randomized controlled trial). Horizontal bars show medians (all groups) and interquartile range (IQR) (cohort 5 only). Antibody responses to PfCSP by ELISA are reported as the difference in reciprocal serum dilution at which the optical density (OD) was 1.0 (OD 1.0) between postimmunization and preimmunization sera (net OD 1.0) for cohorts 1 to 4 (**A**) and cohort 5 (**B**). Results for the PfSPZ automated immunofluorescence assay (aIFA) are the difference in reciprocal serum dilution at which arbitrary fluorescence units (AFU) was 2×10^5 between postimmunization and preimmunization sera (net AFU, 2×10^5) for cohort 5 (**C**). Results for the PfSPZ automated inhibition of SPZ invasion (aISI) assay are the difference in reciprocal serum dilution at which the inhibition of PfSPZ invasion was 80% between postimmunization and preimmunization sera (net inhibition of SPZ invasion 80%) for cohort 5 (**D**). Infection status is based on results of TBS microscopy within 24 weeks after the third vaccination, with unfilled circles representing infected participants and filled (red) circles representing uninfected participants. Horizontal bars show median values and IQR. *P* values are from Brunner-Munzel tests comparing distributions of antibody responses between vaccinees and controls and between infected and uninfected vaccinees. One participant from cohort 2, one from cohort 3 (A), and one from cohort 5 (placebo group) (B to D) are not represented because they did not contribute to post-baseline antibody results, and one outlier in cohort 5 (placebo group) was excluded.

Two methods were used to estimate VE in this trial. The primary efficacy endpoint was based on the RR at 6 months of follow-up. The HR method was also included as an endpoint to facilitate comparison to results of other malaria vaccine trials, which have primarily used HR to calculate VE. The estimated VE of 38% (95% CI, 6.8 to 68.7%) by the RR method means that the vaccine reduced the number of people experiencing at least one infection by TBS within 6 months of follow-up by 38%. The estimated VE of 48% (95% CI, −2.9 to 73.6%) by the HR method means that the time to first infection was longer for vaccinees than controls, although this estimate was not significant ($P = 0.061$).

The results of VE at the end of 6 months of 38% by the RR and 48% by the HR method demonstrated that a three-dose regimen could achieve VE comparable to or perhaps better than achieved with a five-dose regimen (*9*). The 18-month RR VE (15%) is lower and not significant because numbers of first infections during the second malaria season were similar between the two arms (Fig. 1), suggesting a temporal decline in the proportion of the first infections prevented by the vaccine. VE assessed by the HR method was similar for the 6- and 18-month intervals (48 and 46%, respectively). For these analyses, the 18-month estimate was significant ($P = 0.018$), but the 6-month estimate was not ($P = 0.061$). The similar HRs for the two intervals suggest that the proportional hazard assumption was met, as do diagnostic plots. Therefore, although we did not find a significant reduction in the probability of infection over 18 months, we found that the first

infections occurred later among vaccinees over this interval. It is possible that efficacy by this method was significant over the 18-month interval and not the 6-month interval because of higher power from the larger number of events over the longer interval, combined with the fact that the estimated HRs for the two intervals were similar. VE by the HR method has become the standard for assessment of VE of malaria vaccines, including all field trials of the malaria vaccine RTS,S/AS01 (*23*) and a recent trial of R21 (*24*). Our results suggest durable protection, but this finding needs confirmation in larger trials, particularly because two of our four efficacy estimates did not reach significance. A multiplicity adjustment for the four efficacy estimates was not planned because the 6-month RR approach was prespecified as the primary approach. This means that although type 1 error is controlled for the primary approach alone, the family-wise type 1 error rate for the four tests is not strongly controlled, so the three other efficacy results are hypothesis generating rather than confirmatory.

RTS,S/AS01 has now been shown in an implementation pilot program in 920,000 infants in Africa to have a protective efficacy of 21% against hospitalization with malaria and 30% against severe malaria (*25*). On the basis of these results, it is recommended by the World Health Organization for implementation in children from the age of 5 months to reduce malaria burden (*25*). These efficacy estimates are based on clinical endpoints, whereas this early-phase trial analyzed TBS microscopy. RTS,S/AS01 did not show significant VE against infection in adults in Africa (*26*). Thus, demonstrating protection against infection in malaria-experienced adults is an important step forward for a parasite that infects more than 240 million people annually (*1*).

African adults have a much-reduced immune response to immunization with PfSPZ Vaccine as compared to African children and adults in the United States and Europe (*9*, *14*). In this trial, 72% of vaccinees seroconverted, and the median net OD 1.0 of vaccinees was 2363. In a recent trial in the United States, 1.8×10^6 PfSPZ of PfSPZ Vaccine (two of three of the dose administered in this trial) was administered every 8 weeks as in this study, 100% of subjects seroconverted, and the median net OD 1.0 anti-PfCSP antibodies 2 weeks after the third dose was 16,542 (*27*), which is ~5 times the antibody response achieved in this trial. Immunoregulation caused by lifelong exposure to Pf is likely a cause of this reduced immune response. Achieving significant VE in African adults that typically have poor immune responses to Pf antigens strongly supports our contention that PfSPZ Vaccine will be more effective in African children who generate stronger immune responses (*14*). In addition, studies of antibody function beyond automated inhibition of SPZ invasion (aISI) tested in this trial may illuminate mechanisms of protection (*28*).

In mice and nonhuman primates, protective immunity induced by immunization with radiation-attenuated SPZ is dependent on CD8 T cells and interferon-γ; antibodies can be protective but are not required (*29*–*32*). We think that cellular immunity is similarly required for protection in humans and specifically mediated by tissue-resident T cells in the liver, which we have not been able to assess in clinical trials (*3*, *7*, *33*, *34*). However, in a number of PfSPZ Vaccine studies (*3*, *6*, *7*), vaccinees who did not develop Pf infection had higher levels of antibodies to PfCSP 2 weeks after the last dose of vaccine than did vaccinees who developed Pf infection. This trend occurred in this trial, which also included antibodies to whole PfSPZ (fig. S1). However, uninfected and infected vaccinees demonstrated equivalent serum inhibition of SPZ invasion of hepatocytes. If elevated antibodies were a mechanistic correlate of protection, including antibodies blocking SPZ invasion of hepatocytes, we would expect higher inhibitory activity in protected vaccinees. We propose anti-PfCSP and anti-PfSPZ antibodies as nonmechanistic correlates of protection that indicate successful vaccination at the group level. Nonetheless, because no definitive mechanistic correlative of protection has been established, we will be conducting additional system serology studies beyond aISI to assess functional activity of Fc receptor–mediated elimination of infected hepatocytes to further illuminate the potential role of antibodies in protection (*28*, *35*). Unfortunately, peripheral blood mononuclear cells (PBMCs) collected for this study for cellular immunology analyses were lost because of freezer failure, thus eliminating our ability to make these assessments. However, because all data indicate that tissue-resident T cells in the liver mediate protection (*7*, *33*), assessment of PBMCs is unlikely to provide a sensitive and specific indication of protection.

Demonstration of VE against Pf infection with a three-dose immunization regimen in malaria-experienced adults is a critical step in PfSPZ Vaccine clinical development. Our initial goal is to use PfSPZ Vaccine for seasonal malaria protection for 4 to 6 months in 2- to 12-year-old children in the African Sahel after primary immunization and potentially an annual booster. Our ultimate goal is to develop a vaccine formulation and schedule that has the potential to vaccinate the entire malaria-endemic population to halt transmission and eliminate Pf malaria.

METHODS

Study design

We conducted an open-label dose escalation study followed by a double-blind, randomized, placebo-controlled trial (RCT) of PfSPZ Vaccine at a health center in Balonghin, Burkina Faso. Malaria transmission is seasonal, peaking from June to October. The trial was conducted in accordance with the study protocol; the International Conference on Harmonisation of Good Clinical Practices; the Declaration of Helsinki; and regulatory requirements of Burkina Faso, the University of Maryland, Baltimore (UMB), and the National Institute of Allergy and Infectious Diseases (NIAID). The Burkina Faso ethics committee, the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) Institutional Review Board (IRB), the Burkina Faso Regulatory Authority, the UMB IRB, and the NIAID Office of Regulatory Affairs approved the protocol and provided ethical and regulatory oversight for the clinical trial, conducted under U.S. Food and Drug Administration (FDA) Investigational New Drug (IND) application.

Participants

Study participants were healthy males and nonpregnant females aged 21 to 40 years. Female participants practiced contraception beginning 30 days before to at least 30 days after vaccinations. Exclusion and inclusion criteria are listed in the protocol (see the Supplementary Materials) and at ClinicalTrials.gov, NCT02663700. Balonghin community leaders provided assent, and all participants gave informed consent in written French or, for illiterate persons, translated orally into local language and confirmed by an independent witness.

Randomization and masking

The dose escalation study was open label without randomization. The RCT randomized participants 1:1 to receive PfSPZ Vaccine or placebo (0.9% NS). The randomization sequence was generated by a statistician at the Emmes Company, which was the Statistical and Data Coordinating Center (SDCC) for this trial. This included the sequence of 80 treatment assignments, linked to 80 unique alphanumeric codes ("randomization codes") whose pattern had no association with the assignment. The SDCC created a paper list linking these randomization codes with the treatments and provided this only to the study pharmacist, who stored it in a secure location. All other site staff, clinical investigators, and laboratory staff were blinded to treatment assignment. A coinvestigator enrolled participants into the data system, which assigned participant identification numbers sequentially. After a participant was enrolled, the data system displayed the randomization code to the coinvestigator, who provided this to the pharmacist. The pharmacist then referenced the securely stored list to obtain the treatment assignment associated with this code and prepared the appropriate treatment. PfSPZ Vaccine and NS are colorless and indistinguishable and were prepared in the same volume (0.5 ml) and syringe model.

Procedures

PfSPZ Vaccine contains aseptic, purified, cryopreserved PfSPZ (*36*). Participants received 0.5 ml of vaccine or sterile NS (Hospira, Lake Forest, IL, USA) through a 25-gauge needle by DVI over a few seconds and within 30 min of thawing, administered by a blinded vaccinator not responsible for participant follow-up. In the dose escalation study, participants in cohorts 1 to 4 received two doses of 4.5×10^5, 9×10^5, 1.8×10^6, or 2.7×10^6 PfSPZ, respectively, at 0 and 12 to 14 weeks. Dose escalation followed a predefined safety assessment protocol (see the Supplementary Materials). Antibodies to the PfCSP were assessed by enzyme-linked immunosorbent assay (ELISA) in sera collected 2 weeks after second vaccinations (*11*). Because the highest dose of 2.7×10^6 PfSPZ was safe and the most immunogenic, it was selected for cohort 5.

In the RCT, participants received a 7-day course of 200-mg oral artesunate monotherapy (Guilin, Shanghai, China) via directly observed therapy beginning ~10 days before the first and last vaccinations to clear any parasitemia. Participants were enrolled during March 2017, and PfSPZ Vaccine or NS was administered by DVI at 0, 8, and 16 weeks during the dry season, with all participants completing their final dose by 8 July 2017. Seven-day safety data from each of the first two vaccinations were reviewed by the Data Safety and Monitoring Board (DSMB) before the third vaccination. After dose 3, a TBS (primary efficacy endpoint) and dried blood spot for polymerase chain reaction (PCR) analysis (secondary efficacy endpoint) were obtained every 4 weeks (active surveillance) and when participants presented with malaria symptoms that occurred between visits (passive surveillance). The primary analysis endpoint was 24 weeks after last vaccinations, and participants were followed for an additional 52 weeks for VE analysis (Fig. 2).

Participants were monitored for AEs for at least 30 min and again at 1, 3, 7, 14, and 28 days after each vaccination. A study physician remained onsite at all times for unscheduled visits. Staff recorded solicited AEs for 7 days after each vaccination (table S2) and documented unsolicited AEs throughout follow-up. Staff collected specimens for complete blood count with differential, serum creatinine, and alanine aminotransferase on vaccination days and 7 days later. The study team graded AEs according to U.S. FDA guidelines for vaccine clinical trials (*37*) tailored to local normal reference ranges (table S3).

TBSs were prepared for malaria diagnosis every 4 weeks and when participants presented with fever or other malaria symptoms. Blood (0.5 μl) on Giemsa-stained smears was assessed by microscopy (see the Supplementary Materials for description). TBSs were collected from individuals with malaria clinical signs or symptoms, including axillary temperature ≥37.5°C, or were read immediately; other TBSs were read at the end of each 24- or 52-week observation period. Symptomatic participants positive for Pf received standard treatment with artemether-lumefantrine.

At the first vaccinations, 14 days after the third vaccinations and before the second transmission season (for cohort 5 only), blood was collected to measure immunoglobulin G antibodies to PfCSP by ELISA, antibodies to PfSPZ by automated immunofluorescence assay, and functional activity of sera by aISI assay as described (cohort 5 only) (*11*).

Dose escalation criteria

After administration of the first vaccinations for cohorts 1 and 2, 7 days of safety data were reviewed by the principal investigator and independent safety monitor against preset criteria (table S1) before continuing to cohort 3, with a similar review conducted before continuing to cohort 4. The DSMB reviewed 7-day safety data from all four cohorts and approved the second vaccinations. After these were completed, the DSMB reviewed 28-day safety data from all vaccinations and approved progression to the RCT (cohort 5), which was to receive the most immunogenic and/or the highest dose well tolerated in cohorts 1 to 4.

Outcomes

Safety was addressed by assessing solicited AEs within 7 days after vaccination, severe (grade 3) unsolicited AEs and serious AEs considered related to vaccination within 28 days after vaccination, and serious AEs at any time during follow-up. A secondary objective was to measure antibodies to PfCSP. Exploratory objectives for cohort 5 included VE against Pf infection by microscopy over 24 and 76 weeks.

Statistical analysis

All participants who received any study product were assessed for safety. The primary VE analysis was a PP analysis of cohort 5 participants who received all three doses. Our primary endpoint for the exploratory VE objective was TBS positivity within 24 weeks after the third vaccinations. On the basis of an estimated attack rate of 50% for controls, we calculated that a sample size of 38 participants per arm would provide 80% power to detect 60% VE at a significance level of 0.05.

VE was assessed within 24 and 76 weeks of completing the vaccination regimen and was calculated according to the RR and HR approaches. The RR approach (VE = 1 − RR) at 24 weeks was the primary VE analysis. The RR was calculated on the basis of Kaplan-Meier estimates of survival curves for the treatment arms. Variances in the survival function estimates were calculated with Greenwood's formula (*38*), and the CI for RR was calculated by applying the delta method and inverted to obtain the *P* value. The HR approach, added to the statistical analysis plan before unblinding, used a Cox proportional hazards model to estimate the HR, and VE was calculated as

VE = 1 − HR. A score CI was calculated and inverted to obtain the *P* value. An adjusted VE estimate was obtained from a Cox model adjusting for log-transformed baseline antibody level and age. Because the 24-week RR method was primary, adjustment for multiple testing was not performed.

The following prespecified baseline characteristics were considered for inclusion in the adjusted VE estimate: age, sex, baseline anti-PfCSP antibody value, and baseline positive Pf testing by microscopy collected just before first vaccine injections. These covariates were included in an initial Cox model for time to first positive smear to assess their association with time to parasitemia. Next, we assessed numeric covariates (age and baseline anti-PfCSP value) for linearity with the log hazard function by examining plots of martingale residuals. We considered appropriate transformations for nonlinear relationships. We selected a log transformation for anti-PfCSP titer. We considered a cubic spline for age, but the nonlinear component was nonsignificant, so we left age untransformed. Next, we fit a model including all covariates (transformed as appropriate) and dropped covariates with significance levels greater than 0.10 in this model from the final model. The final model uses the exact method for ties and Wald confidence intervals. We used the same process to create the adjusted model for time to first PCR positive. This model includes log-transformed anti-PfCSP antibody levels as well and dropped age, sex, and baseline malaria status from the final model as *P* values >0.10. To assess the proportional hazard assumption for the treatment groups, we examined log(−log) transformations of the survival curves. We plotted cumulative sums of martingale residuals against the covariate value to assess the proportional hazard assumption for the numeric covariates. Diagnostic plots indicated that model assumptions were satisfied. We also created Kaplan-Meier survival curves and used the log-rank test to assess whether the survival curves differed between treatment and control groups.

All efficacy analyses were repeated for the ITT population, which included all participants in cohort 5. These differed negligibly from the PP analyses (table S9). A tertiary analysis analyzed efficacy against multiple infections based on the incidence rate ratio. Because the outcome is malaria infection by TBS measured monthly and at sick visits, it is not clear when two positive smear results represent different infections. We specified in the statistical analysis plan that two positive smears would be considered different infections if they were separated by a negative smear or if the second positive smear occurred >5 days after a completed course of antimalarial medication since the prior positive. Because this process may not cleanly identify distinct infections and the results were not significant, we omitted them from this paper. All efficacy methods were repeated for PCR results, which will be reported separately.

Antibody levels were measured on the day of vaccination and 14 days after the last dose. Anti-PfCSP antibodies were measured by ELISA for all cohorts, and anti-PfSPZ antibodies were measured by automated immunofluorescence assay and inhibition of SPZ invasion assay for cohort 5 only. Immunogenicity analyses were performed on all participants who met PP criteria for the efficacy analysis and contributed post-baseline antibody data. This population also satisfies ITT criteria because the two people (one in cohort 2 and one in the placebo group in cohort 5) who did not complete vaccinations contributed no post-baseline antibody data, which is an exclusion criterion for the ITT population for immunogenicity. The post-vaccination ELISA and automated immunofluorescence assay results showed one outlier: a control arm participant whose antibody responses resembled the vaccine group responses. No evidence for a testing, labeling, or treatment administration error was found. The main analyses were performed excluding these two outliers. Results from sensitivity analyses including these outliers differed negligibly from the main results and are included in table S7.

Analytic methods for anti-PfCSP results by ELISA are described; the same methods were used to analyze anti-PfSPZ antibodies by automated immunofluorescence assay and aISI. Additive changes from baseline were calculated and are termed "net OD 1.0"; ratios of post-vaccination to pre-vaccination levels were also analyzed ("ratio OD 1.0"). Post hoc Brunner-Munzel tests were performed comparing distributions of net OD 1.0 and ratio OD 1.0 between vaccinees, and controls were performed with the Brunner-Munzel test, an extension of the Wilcoxon-Mann-Whitney test that relaxes the assumption of equal variance in different groups (*39*, *40*). The same test was used to compare net OD 1.0 and ratio OD 1.0 values between infected and uninfected vaccinees to assess whether changes from baseline are associated with subsequent risk of infection (within 24 weeks after the third vaccination). Changes from baseline were compared instead of actual post-vaccination levels to adjust for heterogeneity in exposure during the efficacy follow-up period. Whereas pre-vaccination antibody levels may reflect heterogeneity in natural immunity, they may also reflect heterogeneity in exposure due to geography and/or individual behavior and so may be a proxy for heterogeneity in exposure during the efficacy follow-up period. Previous work from a trial in Mali found changes in antibody levels to be significantly associated with subsequent clinical malaria infection when the actual antibody levels were not (*41*). It is also possible that changes in antibody levels could reflect a biological mechanism relating to vaccine uptake.

Barnard's test was used for post hoc comparisons of seroconversion proportions between vaccinees and controls and between infected and uninfected vaccinees. Seroconversion for PfCSP ELISA was defined as an additive increase from baseline of at least 50 OD 1.0 units and at least a threefold increase from baseline. For automated immunofluorescence assay, seroconversion was defined as an additive increase of at least 150 AFU 2.0×10^5 (net AFU) and a threefold increase from baseline (AFU ratio). For aISI, seroconversion was defined as at least an increase of 10 in the reciprocal serum dilution at which there was 80% inhibition from baseline (net 80% inhibition) and a threefold increase in the reciprocal serum dilution at which there was 80% inhibition (80% inhibition ratio). In calculating fold change, baseline values of zero were imputed to 1. In displays of ratio antibody values in the main text, zero values cannot be displayed on a log scale and so are displayed as 0.1.

Supplementary Materials

This PDF file includes:
Texts S1 and S2
Figs. S1 and S2
Tables S1 to S8

Other Supplementary Material for this manuscript includes the following:
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

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